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Chromatin structure has a strong influence on the regulation of all nuclear processes in which access to DNA is required, such as transcription, replication and DNA repair. We have previously shown that Rad54p, a member of the SWI2/SNF2 family required for the repair of DNA double-strand breaks (DSBs) by homology recombination, is a *bona fide* ATP-dependent chromatin remodeling enzyme, and that the ability of Rad54 to modulate chromatin structure is required for the proper repair of DSBs in a chromatin context.

We had previously observed that Rad54p was stabilized against thermal denaturation by chromatin. Here, we report that this stabilizating effect is real and specific, and it can be attributed to the ability of Rad54p to directly interact with the amino-terminal portion of histones H3 and H4. Interestingly, H3 and H4 amino-terminal domains are targets of post-translational modifications required for DNA repair. Particularly, H3 acetylation by Hat1p, and H4 acetylation by Esa1p have been shown to be critical steps in the repair of DSBs. We found that even though Rad54p discriminately binds H3 and H4 proximal amino-terminal domains, this interactions are not essential for its ability to remodel chromatin and drive the homologous recombination process in our *in vitro* setup.

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Introduction

The genome of eukaryotes is packaged into chromatin. The basic repeating unit of chromatin is the nucleosome, a stable complex of the four core histone proteins and DNA. The DNA wraps ~1.75 times around the core histone octamer, adopting a condensed conformation that is further compacted by inter-nucleosomal interactions. Chromatin structure has a strong influence on the regulation of all nuclear processes in which access to DNA is required, such as transcription, replication and DNA repair. We and others have recently shown that Rad54p, a member of the SWI2/SNF2 family that is required for DNA double-strand break (DSB) repair and homologous recombination in organisms ranging from yeast to human, is an ATP-dependent chromatin enzyme, that utilizes the free energy derived from ATP hydrolysis to actively disrupt histone-DNA interactions [1,2,3].

Rad54p works in conjunction with Rad51p, a eukaryotic homologue of the RecA recombinase, to drive the pairing and recombination of homologous DNA molecules on chromatin. Like other members of the SWI2/SNF2 family, Rad54p is able to translocate and generate torsional stress on chromatin [2,4,5]. This superhelical stress may provide the biomechanical force necessary for the disruption of histone-DNA contacts and the creation of short regions of DNA that are transiently dissociated from the octamer surface, therefore allowing the homologous recombination reaction to take place in a chromatin context [2].

In order to take a more detailed look at the mechanism used by Rad54p, we investigated the possibility that Rad54p directly interacts with both DNA and the histone octamer. Here we report that Rad54p specifically binds the proximal NH_2-terminal region of histones H3 and H4. These results correlate with *in vivo* experiments showing that both histone H3 and H4 are modified in response to DNA damage, and that these modifications are critical for downstream DNA repair events [6,7]. Nonetheless, we have not yet been able to recapitulate the histone NH_2- termini requirement *in vitro*.

Body

We had previously observed that purified recombinant Rad54p was readily denatured at temperatures >30°C, even in the presence of DNA, but was highly stabilized and stimulated by chromatin [2]. To address the possibility that the stabilization effect was non-specific, we monitored the ATPase activity of Rad54p at 30°C or 37°C, in the presence of naked DNA and a non-relevant protein (BSA), or DNA-binding proteins

(RPA or free histones). We found that none of these factors stabilizes Rad54p, and the DNA-stimulated ATPase activity at 37°C is low, comparable to the rate detected in the presence of naked DNA alone. These results indicate that nucleosomal DNA is uniquely able to protect Rad54p from thermal inactivation, suggesting that Rad54p may physically interact with one or more components of nucleosomes.

Histone NH_2-terminal tails are required for protection of Rad54p from thermal denaturation. The fact that Rad54p is stabilized by nucleosomal DNA might reflect a physiologically relevant functional interaction between DNA repair proteins and chromatin components. Histone H3 NH_2-terminal tail and its acetylation by Hat1p are required for efficient DNA DSB repair *in vivo* [6]. Similarly, acetylation of histone H4 at its NH_2-terminal domain by Esa1p is required for DNA repair [7]. One simple explanation for the stabilizing effect that nucleosomal DNA has on Rad54p posits that Rad54p might directly interact with nucleosomes. We therefore decided to test whether Rad54p could be protected from thermal denaturation by "tail-less" chromatin reconstituted with the globular domains of the four core histones (i.e., deletion constructs of the core histone proteins that lack the NH_2-terminal domains).

The globular domains of the core histones form a stable octamer that can be reconstituted into chromatin by a standard salt-dialysis protocol. At 30°C, tail-less chromatin stimulates Rad54p's ATPase activity to a level comparable to that of WT chromatin (i.e., chromatin reconstituted with full-length histone proteins). Nevertheless, when the reaction is performed at 37°C, the rate of ATP hydrolysis in the presence of tail-less chromatin drops to levels comparable to those obtained in the presence of naked DNA. These results suggest that tail-less chromatin is unable to protect Rad54p from thermal inactivation, and that the NH_2-terminal domains of the histone proteins play a crucial role in the stabilizing effect that nucleosomal DNA exerts on Rad54p.

Rad54p specifically interacts with histone H3 and H4 NH_2-terminal tails. Given that histone NH_2-terminal tails are required to protect Rad54p from thermal denaturation, we hypothesized that Rad54p directly interacts with these moieties. In order to test this proposition, we asked whether Rad54p could bind to GST-fusion proteins that harbor yeast histone NH_2-termini. We found that Rad54p interacts with GST-H4 and GST-H3 but not GST-H2A or the GST control. Binding to GST-H2B was not determined. The interaction with both histones H3 and H4 is specific to the proximal half of the NH_2-terminal tail, containing amino acid residues 1-25 and 1-34, respectively.

Chromatin remodeling by Rad54p is independent of histone NH_2-terminal tails. NH_2-terminal domains have been shown to be required for multiple rounds of catalytic chromatin remodeling by the yeast SWI/SNF and RSC complexes [8]. Moreover, the chromatin remodeling and ATPase activities of ISWI-containing complexes have been found to require nucleosomal histones for maximal stimulation; specifically, the *Drosophila* NURF complex requires residues 16 to 19 of histone H4 [9,10,11]. In light of these facts, we decided to test whether the histone NH_2-terminal domains are necessary for Rad54p *in vitro* chromatin remodeling activity. To this effect, we performed chromatin remodeling-*Sal*I digestion coupled assays on nucleosomal substrates reconstituted with either full-length recombinant histones, full-length H2A and H2B combined with the globular domains of H3 and H4, full-length H3 and H4 combined with the globular domains of H2A and H2B, or the globular domains of all four core histones. We found that in the absence of Rad54p, the rate of *Sal*I cleavage is very slow for all nucleosomal species. The addition of Rad54p along with Rad51p leads to dramatically enhanced *Sal*I digestion kinetics, regardless of the nature of the nucleosomal substrate.

D-loop formation by Rad51p and Rad54p is independent of histone NH_2-terminal tails. To further characterize the functional interactions between Rad54p and nucleosomal histones, we investigated the ability of Rad54p to facilitate the formation of D-loops by Rad51p on nucleosomal substrates that contained full-length histones, full-length H2A and H2B combined with the globular domains of H3 and H4, full-length H3 and H4 combined with the globular domains of H2A and H2B, or the globular domains of all four core histones. We found that Rad51p, in the presence of Rad54p, proficiently catalyzes the pairing of a supercoiled, double-stranded DNA molecule and a homologous, single-stranded oligonucleotide that is radioactively labeled. The efficiency of this reaction is similar for naked dsDNA substrates, chromatin substrates containing full-length versions of all four core histones, tail-less chromatin substrates containing the globular domains of all four core histones, H3, H4 tail-less chromatin substrates, or H2A, H2B tail-less chromatin substrates.

Development of new in vitro systems to study homologous recombination. Since the systems used in these studies prevented us from detecting subtle differences in kinetics of substrate binding and release, we designed and developed a new setup for investigating the role of histone amino-termini in homologous recombination, based on the sensitive chromatin immunoprecipitation (ChIP) technique. In this system, purified

proteins are detected when bound to a specific chromatin substrate, which will allow us to precisely follow the course of events during the homologous recombination reaction.

Key Research Accomplishments

- Obtained data supporting the proposition that Rad54p directly interacts with histone components of chromatin, and established that this interaction is specific for the proximal portion of the amino-terminal domains of histones H3 and H4
- Obtained results indicating that histone amino-termini are not required for basal Rad54p activity in two functional *in vitro* assays
- Developed a new *in vitro* assay for studying protein-protein and protein-DNA interactions during homologous recombination reactions

Reportable Outcomes

Degrees Obtained

Mariela Jaskelioff, Ph.D. in Biomedical Sciences

University of Massachusetts Medical School, August 2003

Publications

Jaskelioff M. and Peterson, C.L. (2003). Chromatin and transcription: histones continue to make their marks. *Nat Cell Biol* (5):395-9.

Invited Seminars

"Role of Histone NH_2-terminal Domains in DNA Repair by Homologous Recombination". Pennsylvania State University 22nd Summer Symposium in Molecular Biology, "Chromatin Structure and Function". University Park, PA, August 2003

Conclusions

Our results indicate that Rad54p specifically interacts with the proximal portion of histone H3 and H4 NH_2-terminal domains. The direct contact with histone tails emanating from the nucleosome core could account for the ability of nucleosomal DNA to protect Rad54p from thermal denaturation. We have shown that the ability of Rad54p to enhance the accessibility of nucleosomal sequences is independent of its interaction with histone NH_2-terminal domains. This result is not surprising, since previous studies with yeast SWI/SNF and RSC showed that under similar assay conditions, these

complexes efficiently remodel tail-less chromatin [8]. We also report that Rad54p does not require histone NH_2-terminal tails to facilitate the Rad51p-driven strand invasion and D-loop formation reaction on chromatin substrates. The core histone NH_2-terminal tails might influence a step of the reaction that occurs after nucleosome remodeling but prior to intermolecular transfer of the remodeling complex to new nucleosomal arrays. In order to pursue this possibility, we have developed a new assay.

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Chromatin and transcription: histones continue to make their marks

Mariela Jaskelioff and Craig L. Peterson

Chromatin architecture is modulated by a large number of enzymes, resulting in the proper regulation of transcription, replication, cell cycle progression, DNA repair, recombination and chromosome segregation. The structure, regulation and coordination of these enzymatic activities were the main topics of discussion at The Enzymology of Chromatin and Transcription Keystone Symposium held in Santa Fe, NM (March 10–16, 2003).

Chromatin fibres are the natural substrates for all DNA-mediated processes, and thus it is not too surprising that a variety of evolutionarily conserved enzymes modulate the architecture of chromatin, allowing the precise temporal and spatial regulation of genetic processes. These enzymes, the focal point of this Keystone Symposium organized by Shelly L. Berger (Wistar Institute, Philadelphia, PA) and Jerry L. Workman (Pennsylvania State University, State College, PA), can be divided in two major classes: first, those that introduce covalent modifications on histone amino- or carboxy-terminal 'tail' domains; second, those that use the free energy derived from ATP hydrolysis to actively disrupt nucleosomal structure.

With the exception of one plenary session devoted to the mechanism of action of the ATP-dependent remodelling enzymes, many of the presentations centred on the coordinated action of histone-modifying enzymes during the transcription process and the various roles that the subsequent histone 'marks' play in establishing on or off states of gene expression.

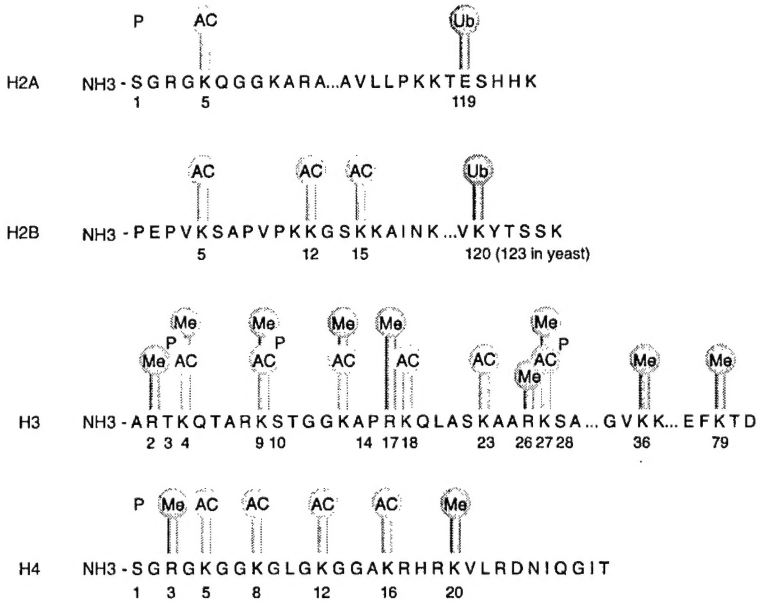


Figure 1 **Potential sites of post-translational modification on nucleosomal histones. Many modification patterns have been closely linked to unique biological outcomes. Ac, acetylation; Me, methylation; P, phosporylation; Ub, ubiquitination.**

Histone methylation comes to centre stage

In his keynote address, C. David Allis (University of Virginia, Charlottesville, VA) proposed that histones are major carriers of epigenetic information and that covalent modifications on the histone N-terminal tails function as master on/off switches that determine whether a gene is active or inactive. Histone tails are subject to a wide range of post-translational modifications (Fig. 1). Although in past years, histone acetylation on lysine residues has received the lion's share of attention, remarkably, histone acetylation was not a major topic of discussion at this meeting. In its place, histone methylation permeated nearly every session. Methylation can have multiple effects on chromatin function, depending on the specific lysine and the level of modification (that is, mono-, di-, or tri-methylation of a single lysine). For instance, H3-K9 di-methylation and H3-K27 tri-methylation are both largely associated with gene silencing and heterochromatin formation[1], whereas methylation of H3-K4, H3-K36, or H3-K79 is associated with active chromatin. Tony Kouzarides (University of Cambridge, Cambridge, UK) also presented evidence that the number of methyl groups on a single lysine is associated with distinct chromatin states. Whereas several inactive genes are marked by H3-K4 di-methylation, the transition to transcriptional competence correlated with H3-K4 tri-methylation[2]. Thus, it is clear that the site specificity of lysine methylation, as well as the number of methyl groups attached to a particular lysine, can have distinct consequences for transcription.

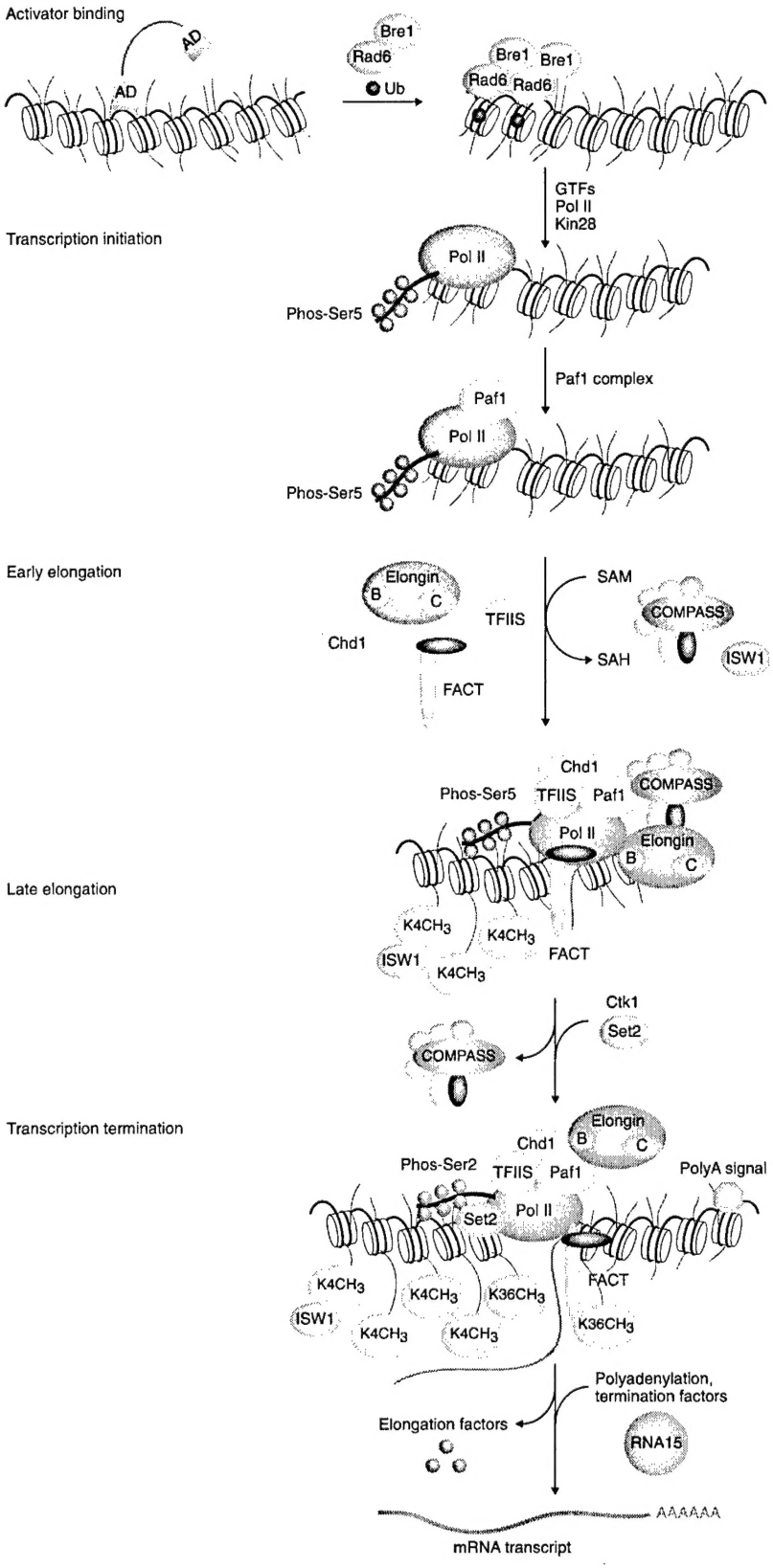
With few exceptions, histone methylation is catalysed by proteins that contain a conserved SET domain flanked by cysteine-rich pre-SET and post-SET domains. Steve Gamblin (Institute for Medical Research, London, UK), Raymond Trievel (NIH, Bethesda, MD) and Xing Zhang (Emory University, Atlanta, GA) each presented crystallographic structures of the catalytic domains from three different methyltransferases — SET7/9, LSRT and DIM-5, respectively — in complex with their substrate. A hallmark of each structure was a narrow, doughnut-like hole at the catalytic core where methyl transfer occurs. The peptide substrate binds on one side of the channel and the methyl donor occupies the opposite face. Once the lysine is docked into

the active site, one hydrogen from the ε-NH_3 protrudes through the hole to the opposite face where the transfer reaction takes place. In the case of a mono-methylase, SET7/9, the target ε-NH_3 group is rigidly bound in the active site such that only a single methyl-transfer event can occur[3]. In contrast, the ε-NH_3 group has greater freedom of rotation in the active site of di- or tri-methyltransferases (LSMT, DIM-5), which allows for additional methylation events[4,5]. Remarkably, Xing Zhang (Emory University) demonstrated that a tri-methylase or a mono-methylase could be converted to a di-methylase simply by changing one of the amino acids that controls the hydrogen-bonding environment of the ε-NH_3 group.

Combinatorial histone modifications: regulating the marks

To date, there are still no reports of a *bona fide* 'demethylase', and thus it seems that histone methylation is a relatively stable chromatin mark that can only be lost by successive rounds of DNA replication or by replication-independent histone replacement. David Allis suggested that cells may use reversible serine/threonine phosphorylation to regulate the biological outcomes of lysine methylation[1]. His 'methyl/phospho switch' hypothesis argues that a phosphorylation event on a histone tail would regulate the binding of an effector protein to an adjacent methylated residue. In support of this model, he presented numerous examples in which a serine or threonine is found adjacent to a methylation site (lysine) throughout the sequence of histone proteins. As a proof of principle, David Allis presented evidence that phosphorylation of Ser 10 of a histone H3 peptide blocks the ability of heterochromatin protein 1 (HP1) to bind its cognate substrate, di-methylated H3-K9.

Whereas the control of histone methylation by adjacent phosphorylation is a newly emerging paradigm, other types of combinatorial histone modifications are better established. For instance, several presentations, including those from Mary Ann Osley (University of New Mexico, Albuquerque, NM), Karl Henry (Wistar Institute, Philadelphia, PA), Brad Bernstein (Harvard Medical School, Boston, MA) and Ali Shilatifard (St. Louis University, St. Louis, MO) demonstrated that ubiquitination of H2B-K123 is required for subsequent methylation of H3-K4 and H3-K79 (refs 6–8). All three of these marks are important for transcriptional activity. Similarly, Robert Roeder (Rockefeller University, New York, NY) presented evidence from *in vitro* studies for a multistep model for p53-mediated *in vitro* transcription, in which methylation of H4-R3 by protein arginine methyltransferase 1



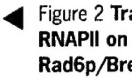


Figure 2 **Transcriptional elongation by RNAPII on chromatin substrates. Rad6p/Bre1p are recruited by transcriptional activators to ubiquitinate H2B. The RNAPII CTD repeats are phosphorylated on Ser 5 by Kin28p. As the RNAPII complex progresses, Ser 5 is dephosphorylated and Ser 2 is phosphorylated by Ctk1p. The H3 K36-specific methyltransferase Set2p associates with the ongoing complex, promoting late elongation. When a polyadenylation site is reached, Ser 2 is dephosphorylated by Fcp1p and most elongation factors dissociate from the RNAPII complex. Polyadenylation and termination factors associate with the RNAPII complex, resulting in the release of the polyadenylated mRNA transcript. We thank Ali 'the fisherman' Shilatifard for help with this figure.**

(PRMT1) stimulates CBP/p300 acetylation of H4-K5, K8, K12 and K16, which in turn promotes the methylation of H3-R2, R17 and R26 by another PRMT family member, CARM1. The integrated action of these histone-modifying enzymes correlates with strong transcriptional activation of the p53-dependent gene. Cellular chromatin immunoprecipitation (ChIP) assays confirmed the stepwise recruitment of these cofactors and accompanying histone modifications in response to activation of a p53-dependent gene by ultraviolet irradiation. Similarly, Michael Stallcup (University of Southern California, Los Angeles, CA) showed that oestrogen-dependent transcription also required the concerted action of PRMT1/CARM1/p300, recruited to the oestrogen-responsive promoter by GRIP-1/ER-activating complex[9]. Finally, Ronen Marmorstein (Wistar Institute, Philadelphia, PA) presented the structural basis for why phosphorylation of H3-S10 enhances the ability of Gcn5p to acetylate H3-K14 *in vitro* and at some sites *in vivo*[10]. He found that the phosphorylated serine induces H3 adjustments both local and distal to H3-S10, resulting in a more optimal anchoring of H3 for K14 acetylation.

How do histone modifications exert their effects?

A large number of presentations focused on how histone marks exert their biological effects. In theory, site-specific histone modifications might influence chromatin function through two mechanisms: first, by affecting nucleosome–nucleosome interactions that control the folding of nucleosomal arrays; second, by promoting or disrupting the chromatin binding of non-histone proteins that link the covalent modification to a biological outcome. Addressing the first potential mechanism has been problematic because of the technical inability to reconstitute nucleosomal arrays that harbour homogeneous and site-specific modifications. In response to this problem, we presented our recent success at developing a native chemical ligation strategy permitting the reconstitution of nucleosomal arrays that harbour a wide range of site-specific histone modifications, including serine phosphorylation, lysine acetylation and lysine methylation[11].

A great deal of progress is being made in the identification of proteins that bind to histone tails harbouring specific marks. Several talks discussed the binding of the heterochromatin component HP1 to the H3 tail dimethylated at K9. Similarly, Yi Zhang (UNC Chapel Hill, NC) presented *in vivo* and *in vitro* studies demonstrating that the *Drosophila melanogaster* and mammalian Polycomb complexes interact with histone H3 tri-methylated at K27. In both of these cases, the binding of effector proteins to methylated H3-K9 or H3-K27 promoted gene silencing[12]. Chromatin marks that are associated with transcriptionally active genes may also exert their effects by influencing the binding of chromatin remodelling factors. Although not discussed at this meeting, acetylated lysines can interact with bromodomains found within subunits of SWI/SNF-like chromatin remodelling complexes[13]. Additionally, Tony Kouzarides presented compelling evidence that methylation of H3-K4 promotes the binding of the yeast Isw1p ATPase. Thus, histone modification provides a means to recruit enzymes that disrupt chromatin structure (for example, Isw1p) or to recruit proteins that further package the chromatin fibre into inaccessible states (for example, HP1).

Heterochromatin formation and silencing

The establishment and maintenance of transcriptional silencing by heterochromatic structures was discussed at length. Michael Grunstein (UCLA, Los Angeles, CA) provided evidence supporting a stepwise model for telomeric heterochromatin assembly in budding yeast. His results indicated that the Rap1p protein, which is localized to the T_{1-3} telomeric repeats, recruits Sir4p, which in turn recruits the histone-binding protein Sir3p and the histone deacetylase Sir2p. The NAD^+-dependent deacetylation of H4-K16 by Sir2p then allows the spreading of these three Sir proteins from the telomere[14]. Work presented by both Michael Grunstein and Masami Horikoshi (University of Tokyo, Tokyo, Japan) indicated that the advancement of this silencing structure into euchromatic regions is blocked by the action of Sas2p, a histone acetyltransferase that targets H4-K16 and counteracts the action of Sir2p[15,16]. In fact, Sas2p seems to have a global role in the maintenance of H4-K16 acetylation. Ann Ehrenhofer-Murray (Max-Planck Institute, Berlin, Germany) reported that Sas2p is part of the yeast SAS-I complex, which interacts with the chromatin assembly factor CAF-1 and is recruited to DNA replication forks to re-establish H4-K16 acetylation on newly assembled chromatin[17].

A number of speakers discussed the roles of HP1 in gene silencing, heterochromatin formation and chromosome segregation. In *Drosophila*, HP1 is associated with pericentric and telomeric regions, a region along the arm of the 4th chromosome and approximately 200 other euchromatic sites. *In vitro*, HP1 prefers to bind to a histone H3 tail dimethylated at K9, and *in vivo* HP1 generally (but not always) colocalizes with this histone mark. Lori Wallrath (University of Iowa, Iowa City, IA) demonstrated that the artificial tethering of a LacI–HP1 fusion protein to an ectopic location could cause the long range silencing of reporter genes[18]. Remarkably, the tethered HP1 did not result in H3-K9 methylation. Sally Elgin (Washington University, St. Louis) then discussed the fact that HP1 requires other gene products to effect heterochromatin-based gene silencing, and that one of these factors, HP2, colocalizes with HP1 at many loci[19]. This HP1-based silencing system is not used solely for establishment of heterochromatin domains, as Frank Rauscher III (Wistar Institute, Philadelphia, PA) provided an example of HP1 recruitment to specific promoters by the KAP-1 corepressor. KAP-1 coordinates histone deacetylation, histone methylation and HP1 deposition to effect silencing by the KRAB-ZFP superfamily of transcriptional repressors. Interestingly, this highly localized silencing structure was mitotically stable and was maintained for more than 50 population doublings, even in the absence of the DNA-bound KRAB repressor[20].

Perhaps the most highly anticipated talks of the symposium were those presented by Shiv Grewal (CSHL, Cold Spring Harbor, NY) and Robin Allshire (Wellcome Trust Centre for Cell Biology, University of Edinburgh), who discussed their recent studies on the role of the RNA interference (RNAi) machinery in establishing and maintaining heterochromatin structures at the fission yeast mating-type locus and centromeres[21,22]. Both groups demonstrated that a short piece of centromeric repetitive DNA was sufficient to establish RNAi-dependent, heterochromatin-based gene silencing at an ectopic location. Furthermore, RNAi was shown to be required to recruit the histone H3-K9 methyltransferase, Clr4, the fission yeast homologue of HP1, Swi6 and cohesion pro-

teins. Shiv Grewal then described recent work indicating that the fission yeast homologue of the Sir2 deacetylase is also required for establishing heterochromatin-based gene silencing at centromeres and the mating-type locus. Robin Allshire also presented data indicating that repression of some euchromatic genes may be regulated by the RNAi machinery/Clr4/Swi6. Thus, it seems that the RNAi-dependent establishment of heterochromatin may represent a more general means for creating silent states at euchromatic loci.

The transcriptional cycle

In contrast to previous meetings of this kind, very few presentations focused on the regulated formation of the transcription pre-initiation complex, but rather transcriptional elongation by RNA polymerase II was the centrepiece of discussion. Work from a number of groups indicated that transcriptional elongation involves an enormous number of regulatory factors (Fig. 2). As expected, several talks focused on the how RNAPII contends with the nucleosomal barrier to elongation. Danny Reinberg (UMDNJ, Piscataway, NJ) presented compelling evidence for association of the FACT complex with elongating polymerase in *Drosophila* (in collaboration with J. Lis) and yeast cells. Mechanistic studies by his group (and in collaboration with V. Studitsky) indicated that FACT facilitates nucleosomal elongation by destabilizing one of the histone H2A/H2B dimers. Robert Kingston (Harvard Medical School, Boston, MA) also presented *in vivo* evidence that the human SWI/SNF ATP-dependent remodelling complex is recruited to the hsp70 promoter by heat shock transcription factor, where SWI/SNF is then central to transcriptional elongation.

Several presentations focused on the role of multisite phosphorylation of the heptapeptide repeats in the C-terminal domain (CTD) of the largest subunit of RNAPII as a means for recruiting various elongation factors. For instance, Stephen Buratowski (Harvard Medical School, Boston, MA) discussed how phosphorylation of Ser 5 within the CTD of early elongation complexes results in recruitment of the mRNA-capping enzyme and the Set1p-containing histone H3-K4 methyltransferase complex, COMPASS (Fig. 2). Similarly, Ali Shilatifard showed that recruitment of COMPASS to this elongating form of RNAPII involved its interaction with the PAF1 complex, which is known to associate with the elongating form of RNAPII. He also showed that the PAF1 complex controls the histone methyltransferase activity of Dot1p, thereby linking methylation of H3-K79 to transcriptional elongation[23] (See News and Views by Bryan Turner on page 390 of this issue). Work presented by Tony Kouzarides and Antonin Morillon (University of Oxford, Oxford, UK) indicated that the elongation role of the COMPASS methyltransferase complex may involve recruitment of the Isw1p ATP-dependent chromatin remodelling enzyme, as Isw1p binds to an H3 tail that is di- or tri-methylated at K4, and *ISW1* is required *in vivo* for a normal distribution of RNAPII on coding regions. As elongation by RNAPII progresses, Ser 2 on the CTD is phosphorylated by the Ctk1p kinase. Results presented by both Brian Strahl (UNC, Chapel Hill, NC) and Ali Shilatifard indicated that phosphorylation of Ser 2 may release the COMPASS complex and result in recruitment of Set2p, which methylates H3-K36. Why H3 methylation sites change during the stages of RNAPII elongation is not known.

Ubiquitin-dependent proteolysis and transcription

Several presentations described examples of transcriptional regulation by ubiquitin-dependent recruitment of proteosomal components. Talks by Thomas Kodadek (UT Southwestern, Dallas, TX) and Michael Hubner (EMBL, Heidelberg, Germany) described how the functioning of either yeast Gal4p or the mammalian oestrogen receptor are controlled by ubiquitin-dependent recruitment of the 19S APIS proteasomal subcomplex. In the case of Gal4p, Kodadek presented evidence that Gal4p may be ubiquitinated when bound at the *GAL1* promoter, and that subsequent recruitment of a 19S APIS proteasomal subcomplex might regulate Gal4p DNA binding or control Gal4-RNAPII holoenzyme interactions[24]. Hubner presented an integrated model for the cyclic turnover of oestrogen receptor α on responsive promoters. In this model, both unliganded and liganded oestrogen receptor α, as well as E3 ubiquitin ligases and the 19S APIS complex, cycle on and off an oestrogen response element *in vivo*. Subsequent ubiquitination and proteasome-mediated removal of oestrogen receptor α from the promoter are instrumental for maintaining oestrogen signalling. Consequently, inhibition of the proteasome results in the loss of oestrogen receptor α-mediated transcription, whereas transcriptional inhibition prevents oestrogen receptor α degradation by the proteasome[25]. Thus, both of these studies suggest that dynamic regulation of activator degradation or function may control transcriptional activation.

Transcriptional regulation by ubiquitin-dependent events does not seem to be restricted to the gene-specific activators. Jesper Svejstrup (Cancer Research UK London Research Institute, London, UK) discussed their identification of a yeast factor called DEF1, which seems to be a novel elongation factor that is required for ubiquitin-dependent degradation of RNAPII in response to DNA damage[26]. On a related note, Joan Conaway (Stower Institute, Kansas City, MO) described their ongoing studies characterizing the Elongin B/C ubiquitin ligase complex. She described yeast two-hybrid and biochemical studies, which show that Elongin B/C interacts directly with the Med8p subunit of the mammalian mediator complex. In fact, she showed that purified preparations of mammalian mediator contain sub-stoichiometric levels of Elogin B/C and that mediator has ubiquitin ligase activity[27]. What the targets for this activity might be in the transcription initiation or elongation complex are not known.

The future

As researchers continue to pry their way into the mysteries of chromatin and transcriptional control, the possibilities for regulation seem endless. A multitude of histone modifications control recruitment of chromatin remodelling enzymes, as well as proteins that influence the higher-order folding of chromatin fibres. It now seems that millions of Daltons of protein are not only bound at the transcription initiation site, but that even larger protein assemblies travel down the gene with the elongating polymerase. And yet, although the meeting described many new factors and new regulatory paradigms, many old topics went virtually unmentioned. For instance, linker histones and histone variants are general features of the intrinsically heterogeneous chromatin fibre, but almost no mention was made of how these factors control the solution state behaviour and function of chromatin fibres. Recent *in vivo* studies from Belmont and colleagues indicate that transcription actually occurs on enormous, 100–400-nm thick chromatin fibres[28]. How can this fibre accommodate the many proteins involved in the initiation and elongation of transcription? And finally, as we noted at the start of this report, chromatin structure and histone modifications are commonly thought to imprint an epigenetic code on transcriptional patterns. However, we still have little knowledge of how chromatin states are propagated after passage of a replication fork. It is clear that we still have lots of work to do before all of the mysteries are solved. □

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